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ORIGINAL ARTICLE

Detection and molecular characterization of VRE isolates in Slovakia from stool samples positive for *Clostridioides difficile* toxins

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Abstract

The study aimed to identify colonized patients as a possible source of eventual VRE (vancomycin-resistant enterococci) infection from stool samples positive for glutamate dehydrogenase antigen, as well as for *Clostridioides difficile* toxins A and B. The study was carried out from 7/2020 to 9/2021. Stool samples were grown in a brain heart infusion medium with a gram-positive non-spore-forming bacteria supplement under aerobic conditions. The samples for VRE identification were grown on CHROMID® VRE agar, and the MICs for vancomycin and teicoplanin were also estimated. The presence of the *van*A/*van*B genes was tested using the PCR method. The total number of 113 stool samples positive for *Clostridioides difficile* toxins was analyzed. Of these samples, 44 isolates with VRE characters were identified. The most prevalent isolates in our set of isolates were *Enterococcus faecium* (27 isolates, 62%), *Enterococcus faecalis* (9 isolates, 21%), *Enterococcus solitarius* (4 isolates, 9%), *Enterococcus durans* (2 isolates, 4%), 1 isolate *Enterococcus sulfurous* (2%), and *Enterococcus raffinosus* (2%). In total, 26 isolates were detected in the study in the presence of *vanA* genes (24 isolates *E. faecium*, 2 isolates *E. faecalis*) and 18 isolates detected in the presence of *vanB* genes (7 isolates *E. faecalis*, 4 isolates *E. solitarius*, 3 isolates *E. faecium*, 2 isolates *E. durans*, 1 isolate *E. sulfurous*, and *E. raffinosus*). The results of this study showed the local dominance character of the *vanA* gene of hospital VRE isolates that were carriers of genes associated with high resistance to vancomycin, teicoplanin, and occasionally linezolid.

Keywords Vancomycin-resistant enterococci · Antibiotic susceptibility testing · Genotype · PCR

Introduction

Enterococci are part of the normal intestinal community but are also one of the main causes of opportunistic infections. Enterococci have intrinsic resistance to commonly used antibiotics and also reveal the ability to acquire transferable resistance to antibiotics. Our publication focuses on the pathogenic potential of vancomycin-resistant enterococci (VRE). Enterococci can colonize the gastrointestinal tract, skin, and urine system without clinical symptoms. However, if the isolates become resistant to antibiotics, these bacterial isolates can cause severe infections, especially in people after surgical operation, patients with immunodeficient status, or other infections. Currently, it is not rare to isolate VRE isolates from patients with the character of nosocomial infection. Enterococci can cause sepsis, abscesses, colitis, wound infection, pneumonia, endocarditis, meningitis, and urinary tract infection. (Rangberg et al. 2019; Agegne et al. 2018). Usually, the most frequent isolates are *Enterococcus faecium* and *Enterococcus faecalis*. *Enterococcus*

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raffinossus, *Enterococcus avium*, *Enterococcus durans*, and other species have also been identified from clinical specimens but with a lower frequency. VRE isolates have been characterized as isolates with high resistance to a wide range of antibiotics, especially vancomycin (Zaheer et al. 2020). The main problem of VRE therapy is the presence of comorbidities in patients, patients in intensive care units, and cancer patients. These patients receive chemotherapy and/or other types of aggressive therapy. All these processes lead to the breakdown of the natural barrier mechanisms against pathogenic organisms, and eventually, an infection called colonization resistance of the gut microbiome can develop. We know that enterococci are a standard part of the gut microbiota; however, the complete translocation process from commensals to becoming a pathogen is not yet clear (Ramos et al. 2020). Enterococci are blood colonization bacteria with many mechanisms for adaptation to a new environment such as the gastro- or urine tract. The spread of VRE infection has not been apparent; however, as mentioned above, some groups of patients are at increased risk of VRE infection. Predisposing factors to the development of VRE infection are invasive devices such as urinary or central venous catheters and intrathoracic/abdominal surgery (Vehreschild et al. 2019).

Material and methods

Clinical isolates

In our study, stool samples of patients positive for glutamate dehydrogenase antigen (GDH) and *Clostridioides difficile* toxins A and B were analyzed in a period 7/2020–9/2021 at the Central Military Hospital in Ružomberok – Faculty Hospital, Slovakia.

GDH test

Stool samples were tested using an immunochromatographic method for the qualitative detection of the GDH antigen, also for *Clostridioides difficile* toxins A and B, as recommended by the manufacturer (VEDALAB, France). The positive GDH test was used to screen potentially infected patients with *Clostridioides difficile*.

Methods of cultivation

Stool samples positive for the GDH antigen were cultured in brain heart infusion (Himedia, India) with the supplement of gram-positive non-spore-forming bacteria (Himedia, India) under aerobic conditions for 48 h at 37 °C. The samples for VRE identification were grown on CHROMID® VRE agar (bioMérieux, France). The plates were grown in aerobic conditions for 24 h at 37 °C. Samples with negative results after cultivation on CHROMID® VRE were further tested for vancomycin-sensitive enterococci (VSE) isolates. The samples were grown on Bile esculin agar (Himedia, Czech Republic) with vancomycin 30-µg disk overnight at 37 °C.

Identification of isolates

Colonies after culture on CHROMID® VRE agar were further recultured on Columbia blood agar (Trios s.r.o., Czech Republic) to prevent potential interaction with biochemical substances of chromogenic agar. Plates were grown under aerobic conditions overnight at 37 °C. The diagnostic process was carried out using conventional laboratory methods, including gram staining, antibiotic susceptibility, and biochemical testing. For the final identification of isolates, an EN-COCCUS test was used based on instructions and the identification program recommended by the ErbaExpert manufacturer (Erba Lachema, Czech Republic). The reliability of the identification based on biochemical testing was greater than 99%.

Antibiotic susceptibility testing

The antibiotic susceptibility testing of VRE isolates was tested using the disk diffusion method. For the antibiotic sussceptibility testing were used: penicillin 6 µg (Bio-Rad, Czech Republic), vancomycin 30 µg just for VSE detection, tetracyclin 50 µg, teicoplanin 30 µg, linezolid 10 µg, nitrofurantoin 50 µg, streptomycin 25 µg, chloramphenicol 50 µg, clindamycin 10 µg, erytromycin 30 µg, trimethoprim/sulphamethoxazole 25 µg (Oxoid, Czech Republic). The MICs of vancomycin and teicoplanin of the isolates were estimated using the E test method (0–256 µg/mL (bioMérieux, France).

Detection of vanA and vanB genes

The *vanA*/*vanB* genes were detected in 2–5 colonies (based on the size of the colonies) of VRE isolates cultured on Columbia blood agar. These colonies were mixed with PCR water and Chelex 100 (Bio-Rad, Czech Republic), followed by boiling at 80–90 °C for 10 min. After boiling, the samples were centrifuged at 10,000 rpm for 10 min. The supernatant was used for gene detection. For the detection of genes, we used a sequence synthesized and designated by East Port Prague: (*vanA*1 GGGAAAACGACAATTGC and *vanA*2 GTACAATGCGGCCGTTA, 732 bp) and (*vanB*1 ATG GGAAGCCGATAGTC *vanB*2 GATTTCGTTCCTCGACC, 635 bp) in combination with a master mix including Taq polymerase, PCR buffer, and primers were purchased from Promega (East Port LifeScience, Czech Republic). A PCR program was established on initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 1 min, annealing at 54 °C

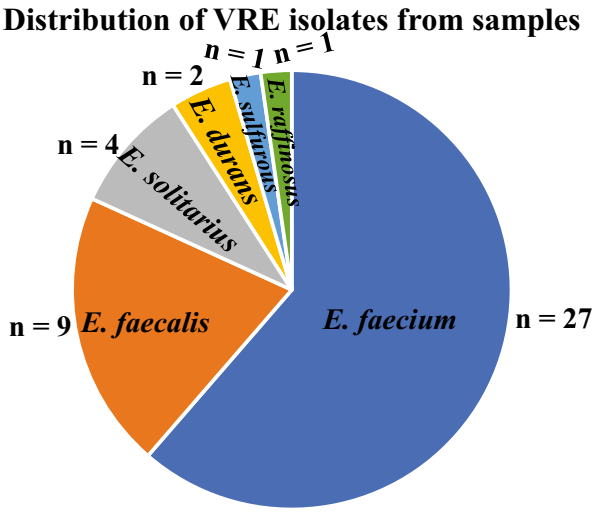

Fig. 1 Distribution of VRE isolates based on biochemical identification

for 1 min, and extension at 72 °C for 1 min. The program was made up of 30 cycles. Electrophoresis was performed for 1 h at 100 V on 1% agarose gel (Bio-Rad, Czech Republic) (Özsoy and Ilky 2017). The gel after electrophoresis was visualized by ethidium bromide solution, and the results were detected under a transilluminator.

Statistical analysis

The statistical analysis distributions of the *vanA* and *vanB* genotypes (in the cases of the presence of both genotypes of isolates) were performed using SPSS 21.0 to determine the significance of ANOVA, a one-way analysis of variance with significance at the level $p < 0.05$.

Results

During the period 7/2020–9/2021, stool samples of patients were collected for further analyzes. We detected 113 stool samples positive for *Clostridioides difficile* toxin. From this number, 44 and 31 were positive for VRE and VSE using CHROMID® VRE and Bile esculin agars, respectively. In the VSE group, 68% (21 isolates) of *E. faecium* and 32% (10 isolates) of *E. faecalis* were detected. Among VRE isolates (Fig. 1), the most prevalent were *E. faecium* (27 isolates; 61.3%), *E. faecalis* (9 isolates; 20.4%), *E. solitarius* (4 isolates; 9.0%), *E. durans* (2 isolates; 4.5%), *E. sulfurous* (1 isolate, 2.2%), and *E. raffinosus* (2.2%). All of these isolates were tested for antibiotic susceptibility and analyzed for the presence or absence of *vanA*/*vanB* genes. For the VRE isolates, the MIC was established for vancomycin (VAN, Table 1) and teicoplanin (TEI, Table 2). As many as 41 VRE isolates showed the MIC for VAN greater than 256 µg/mL (92%). Three isolates reached the MIC of 192 µg/mL (6%) and one 128 µg/mL (2%).

From 44 VRE isolates, 16 gained the MIC for TEI of 192 µg/mL and greater. The second group includes isolates with a MIC of 128 µg/mL (3 isolate *E. faecium* and 1 isolate *E. faecalis*) and the third with a MIC of 96–64 µg/mL (5 isolates of *E. faecium*). However, most of the VRE isolates showed a MIC of 16–1 µg/mL (3 isolates *E. faecalis*, 4 isolates *E. solitarius*, 2 isolates *E. durans*, 1 isolate *E. raffinosus*, 1 isolate *E. sulfurous*, 1 isolate *E. faecalis*).

We detected 18 isolates with the presence of the *vanB* genotype (7 isolates *E. faecalis*, 4 isolates *E. solitarius*, 3 isolates *E. faecium*, 2 isolates *E. durans*, 1 isolate *E. sulfurous*, and *E. raffinosus*) and 26 isolates with *vanA* genotype (24 isolates *E. faecium*, 2 isolates *E. faecalis*). Among

Table 1 VRE isolates are divided into groups according to the susceptibility to VAN (*n* = number of isolates)

Species	*van* gene	MIC VAN			
E. faecium	*vanA*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	24	24	0	0	*n*
	vanB	> 256 µg/mL	192 µg/mL	128 µg/mL	
	3	3	0	0	*n*
E. faecalis	*vanA*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	2	2	0	0	*n*
	vanB	> 256 µg/mL	192 µg/mL	128 µg/mL	
	7	5	2	0	*n*
E. solitarius	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	4	3	0	1	*n*
E. durans	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	2	1	1	0	*n*
E. raffinosus	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	1	1	0	0	*n*
E. sulfurous	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	
	1	1	0	0	*n*

Table 2 VRE isolates are divided into groups according to susceptibility to TEI (n = number of isolates)

Species	*van* gene	MIC TEI					
E. faecium	*vanA*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	24	7	9	3	5	0	***n***
	vanB	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	3	0	0	0	0	3	***n***
E. faecalis	*vanA*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	2	0	0	1	1	0	***n***
	vanB	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	7	0	0	0	0	7	***n***
E. solitarius	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	4	0	0	0	0	4	***n***
E. durans	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	2	0	0	0	0	2	***n***
E. raffinosus	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	1	0	0	0	0	1	***n***
E. sulfurous	*vanB*	> 256 µg/mL	192 µg/mL	128 µg/mL	96–64 µg/mL	16–1 µg/mL	
	1	0	0	0	0	1	***n***

the isolates with the *vanA* genotype character, we observed 26 isolates with MIC for TEI 64 µg/mL or higher. The *vanA* genotype of *E. faecium* (Table 3) manifested resistance against penicillin, chloramphenicol, nitrofurantoin, streptomycin, and trimethoprim/sulfamethoxazole (resistance between 75.0 and 4.1%). We confirmed 2 isolates resistance against linezolid (resistance 8.3%). The *vanB* genotype of *E. faecalis* (Table 4) had resistance levels similar to the *E. faecium vanA* genotype against penicillin at a level of 86.0%. Against other ATBs, the level of resistance ranged from 14.0 to 28.5%.

From the others isolates (Table 5), the most frequent isolate was confirmed than a the *E. solitarius vanB* genotype with resistance against penicillin at the level of 50.0%. The equal status was identified in the *E. durans vanB* genotype (2 isolates). Isolates with confirmation of the *vanA* genotype were not detected. All isolates characterized by the gene in the study are presented in Fig. 2.

We observed significant differences in the distribution between *vanA* and *vanB* genotypes of *E. faecium* ($p < 0.001$), and the same situation was occurred in the case of *E. faecalis* ($p < 0.001$) genotypes.

Table 3 Resistance level of *E. faecium* (n = number of isolates, percent of resistance based on the result of the disk diffusion test)

E. faecium[a]	Total		*vanA*	
ATB	$n = 27$	%	$n = 24$	%
Vancomycin	27	100.0	24	100.0
Penicillin	20	74.0	18	75.0
Linezolid	2	7.4	2	8.3
Teicoplanin	11	40.7	11	45.8
Tetracyclin	10	37.0	10	41.6
Erythromycin	15	55.5	14	58.3
Streptomycin	4	14.8	4	16.6
Sulfomet/Trimet	1	3.7	1	4.1
Clindamycin	14	51.8	14	58.3
Chloramphenicol	3	11.1	3	12.5
Nitrofurantoin	8	29.6	8	33.3

[a]Among the *E. faecium vanB* genotype, a total of 3 isolates were detected. Resistance to a penicillin (2 isolates, 67%) and erythromycin (1 isolate, 33%) was detected

Table 4 Resistance level of *E. faecalis* (n = number of isolates, percent resistance based on the result of the disk diffusion test)

E. fecalis[a]	Total		*vanB*	
ATB	$n = 9$	%	$n = 7$	%
Vancomycin	9	100.0	7	100.0
Penicillin	8	89.0	6	86.0
Linezolid	0	0.0	0	0.0
Teicoplanin	3	33.0	2	28.5
Tetracyclin	2	22.0	2	28.5
Erythromycin	2	22.0	2	28.5
Streptomycin	1	11.0	1	14.0
Sulfomet/Trimet	0	0.0	0	0.0
Clindamycin	2	22.0	2	28.5
Chloramphenicol	1	11.0	1	14.0
Nitrofurantoin	2	22.0	1	14.0

[a]Among the *E. faecalis vanA* genotype, a total of 2 isolates were detected. Both isolates were resistant to vancomycin and penicillin (2 isolates, 100%) and 1 isolate was resistant to teicoplanin and 1 isolate to nitrofurantoin

Table 5 Resistance level *vanA*/*vanB* other isolates (n = number of isolates, percent resistance based on the result of the disk diffusion test)

Others isolates	Total		*vanB*	
ATB	$n=8$	%	$n=8$	%
Vancomycin	8	100.0	8	100.0
Penicillin	4	50.0	4	50.0
Linezolid	0	0.0	0	0.0
Teicoplanin	2	25.0	2	25.0
Tetracyclin	1	12.5	1	12.5
Erythromycin	2	25.0	2	25.0
Streptomycin	1	12.5	1	12.5
Sulfomet/Trimet	1	12.5	1	12.5
Clindamycin	4	50.0	4	50.0
Chloramphenicol	0	0.0	0	0.0
Nitrofurantoin	0	0.0	0	0.0

From the category with gastrointestinal diagnoses (Table 6), we studied 13 clinical isolates. In this group, the mean age was 71 years ± 10.9 SD. The group revealed a sex ratio of 54% women and 46% men. The most frequent diagnosis was infectious gastroenteritis ($n=6$). From patients with gastroenteritis, we characterized isolates with the presence of genotype *vanB* ($n=5$) and 1 isolate with genotype *vanA* of *E. faecium*. These isolates had a similar MIC character to VAN and TEI. From patients with *Clostridioides difficile* enterocolitis, we isolated 2 isolates (*E. faecalis* and *E. solitarius*) with the *vanB* genotype. Genotype with character of *vanB E. faecalis* was also confirmed from patient with acute pancreatitis. *E. faecium* genotype *vanA* was confirmed in cases of ileus, function dyspepsia, and unspecified liver diseases. From this group (Fig. 3), we have confirmed statistical significance beetwen the *vanA* and *vanB* genotype *E. faecium* ($p<0.001$).

From the category (Table 7) with respiratory tract infections and diagnoses, we studied 19 clinical isolates. In this group, the mean age was 72 years ± 19.8 SD and the sex ratio was 90% female and 10% men. The most frequent diagnosis was viral pneumonia ($n=10$), including SARS-CoV-19 ($n=4$). From these patients, we characterized isolates with the presence of *vanA* ($n=5$) and *vanB* ($n=5$) ($n=4$ *E. faecium vanA*; $n=1$ *E. faecium vanB*; $n=1$ *E. faecalis vanA* and *E. faecalis vanB*; $n=1$ *E. durans vanB* and *E. solitarius vanB*). The MIC for VAN varies from 128 to > 256 µg/mL and for MIC TEI, there is a wide interval of 4 to 192 µg/mL. The second most frequent diagnosis was respiratory failure ($n=6$), including 3 cases of acute respiratory failure. From these patients, we characterized isolates with the presence of *vanA* ($n=4$) and *vanB* ($n=2$) ($n=4$ *E. faecium vanA*; $n=2$ *E. faecalis vanB*). The MIC for TEI was 6 to 192 µg/mL, and all isolates gained an MIC for VAN higher than > 256 µg/mL. The last diagnoses from respiratory diagnosis were confirmed as unspecified bacterial pneumonia ($n=2$) and a patient with dyspnea. From group of respiratory tract diagnoses (Fig. 4), we identified statistical significance among the *vanA* and *vanB* genotype *E. faecium* ($p<0.001$), also among the *vanA* and *vanB* genotype *E. faecalis* ($p<0.001$).

The last category of patients (Table 8) is heterogeneous and includes a wide spectrum of chronic and acute diagnoses that could not be classified into the previous categories. In this group, the mean age was 80 years ± 8.7 SD. The group consisted of 54% of women and 46% of men. The most frequent isolate in this group was *E. faecium vanA* genotype ($n=8$) in association with volume depletion, unspecified fever, malignant neoplasm of the bladder wall, iron deficiency anemia, unspecified bacterial infection, heart failure, and gangrene also with ischemic stroke. These isolates had a MIC for VAN higher than > 256 µg/mL. We identified the second most frequent isolate as *E. faecalis vanA* genotype ($n=2$) and 1 isolate *vanB genotype*. In a patient with cerebral infarction due to embolism, *E. faecalis vanB* genotype was confirmed. This isolate had a MIC for TEI of 128 µg/mL and VAN > 256 µg/mL. The last isolates identified in this group were *E. sulfurous* and *E. solitarius*. Both isolates have *vanB* character of genotypes (Fig. 5).

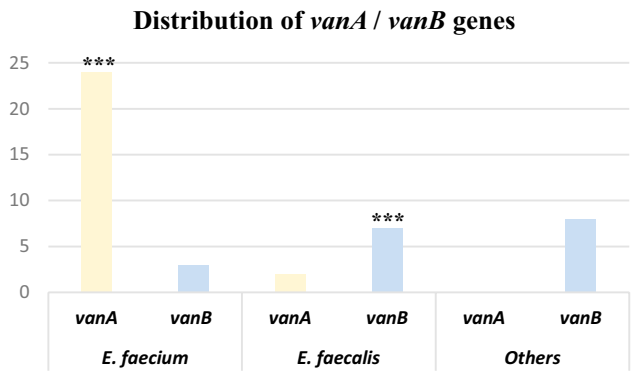

Fig. 2 Distribution *of vanA*/*vanB* genes from isolates ($p<0.001$***) (ANOVA, one-way analysis of variance, SPSS 21.0)

Discussion

The main aim of our study was to identify patients colonized with VRE isolates as a source of eventual VRE infection in the Military hospital from stool samples positive for *Clostridioides difficile* toxins. One limitation of our study may be the not entirely significant taxonomic identification VRE isolates: *E. solitarius*, *E. durans*, *E. sulfurous*, and *E. raffinosus* because of the possible variability of the test results obtained by biochemical tests. However, this result does not change the fact that the isolates belong to VRE

Table 6 List of patients with gastrointestinal diagnosis (diagnosis based on the ICD-10-CM nomenclature) with confirmed VRE isolates (VAN vancomycin, TEI teicoplanin, F female, M male)

Number	Isolates	VAN µg/mL	TEI µg/mL	van genes	Sex	Age	Diagnose
VRE1	*E. faecium*	> 256	> 256	*vanA*	F	82	K30
VRE2	*E. faecalis*	> 256	16	*vanB*	F	63	A04.7
VRE3	*E. faecium*	> 256	3	*vanB*	F	56	A09
VRE4	*E. solitarius*	> 256	4	*vanB*	M	88	A09
VRE5	*E. faecium*	> 256	8	*vanB*	F	64	A09
VRE6	*E. faecalis*	> 256	16	*vanB*	M	70	K85.9
VRE7	*E. durans*	192	3	*vanB*	F	87	A09
VRE8	*E. faecium*	> 256	64	*vanA*	M	56	K56.7
VRE9	*E. raffinosus*	> 256	1	*vanB*	F	81	A09
VRE10	*E. faecium*	> 256	> 256	*vanA*	F	64	A09
VRE11	*E. solitarius*	> 256	12	*vanB*	M	70	A04.7
VRE12	*E. faecium*	> 256	128	*vanA*	M	77	K30
VRE13	*E. faecium*	> 256	> 256	*vanA*	M	69	K76.9

isolates based on the detection of *vanA* and *vanB* genes by PCR. Therefore, we focused on detecting and confirming *vanA* and *vanB* genes by a reliable and conventional PCR detection. However, the biochemical identification of microorganisms still belongs to the relevant and valid identification method in clinical microbiology. For a decrease in VRE infections in healthcare facilities, it is critical to avoid contact between at-risk patients and latent carriers of the VRE infection and avoid the implementation of control steps based only on the knowledge of the latent carrier. Patients with oncological diagnosis, immunodeficient status, organ transplanted, and chronic diseases such as diabetes, having a urinary catheter or intravenous catheter for a long time or taking antibiotics for a long time in hospital care, are exposed to an increased risk of developing severe VRE infections (Wingler et al. 2021; Sivaradjy et al. 2021; Büchler et al. 2022).

The development of VRE and CD infections can occur simultaneously because of the sharing of the same risk factors. Not all enterococcal colonized patients reveal VRE infection; however, they do not always show the clinical signs or clinical manifestations of enterococcal infection. Patients colonized with VRE during CD infection have an increased risk of skin contamination and thus VRE spreading in the hospital environment. VRE infection has the character one of the most important nosocomial diseases that can show an infection process of the bloodstream, intraabdominal, or urinary tract (Sutter et al. 2010; Fujiya et al. 2021). The total count of enterococci bacteria confirmed or without VRE character is similar in the stool samples in the case of colonized and during infection; therefore, it is difficult to determine the exact counts of bacterial pathogens as a limit among asymptomatic colonization and clinical diseases. If a hospital's VRE infection rate is based solely on VRE isolates from clinical samples from patients with infection symptoms, hospitals may be adequately reporting their infection rate but may underestimate their true burden (and therefore the transmissibility/infection) of VRE in hospitals (Chanderraj et al. 2020).

Implementing effective mechanisms for control and transmission to patients with knowledge of the character and genotype of VRE is basic and a foundational step to eradicate the spread of VRE infection in at-risk patients. Our study focused on the presence of the *vanA* and *vanB* genes

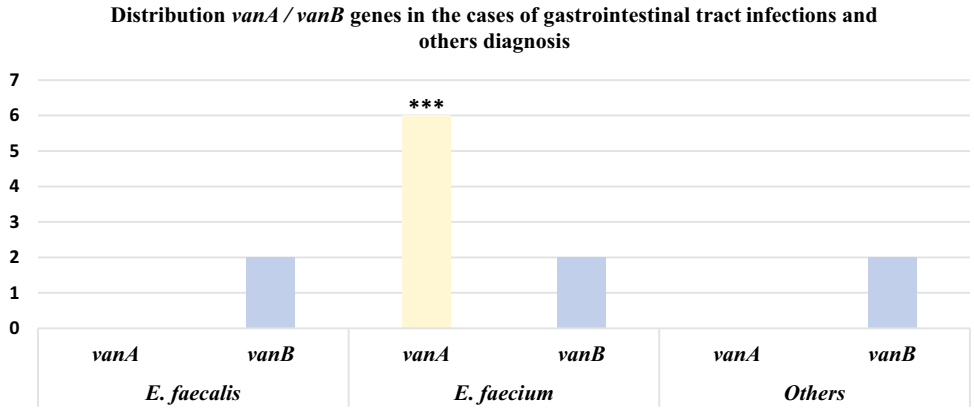

Fig. 3 Distribution *of vanA*/*vanB* genes in cases of gastrointestinal tract diagnosis ($p < 0.001$*** (ANOVA, one-way analysis of variance, SPSS 21.0)

Table 7 List of patients with respiratory tract diagnosis (diagnosis based on ICD-10-CM nomenclature) with diagnosed VRE isolates (VAN vancomycin, TEI teicoplanin, F female, M male)

Number	Isolates	VAN µg/mL	TEI µg/mL	van genes	Sex	Age	Diagnose
VRE1	*E. faecium*	> 256	192	*vanA*	F	79	U07.1
VRE2	*E. faecium*	> 256	192	*vanA*	M	79	R06.0
VRE3	*E. faecium*	> 256	128	*vanA*	F	63	J12.8
VRE4	*E. faecium*	> 256	128	*vanA*	F	74	J96.9
VRE5	*E. faecium*	> 256	192	*vanA*	F	68	J96.9
VRE6	*E. faecalis*	192	12	*vanB*	F	79	J12
VRE7	*E. faecalis*	> 256	4	*vanB*	M	59	U07.1
VRE8	*E. faecalis*	> 256	64	*vanA*	F	83	U07.1
VRE9	*E. durans*	> 256	4	*vanB*	F	85	U07.1
VRE10	*E. faecium*	> 256	192	*vanA*	F	61	J12.8
VRE11	*E. faecium*	> 256	192	*vanA*	F	63	J96.0
VRE12	*E. solitarius*	128	2	*vanB*	F	91	J12.8
VRE13	*E. faecalis*	> 256	8	*vanB*	F	62	J96.0
VRE14	*E. faecalis*	> 256	6	*vanB*	F	67	J96.0
VRE15	*E. faecium*	> 256	192	*vanA*	F	60	J96.9
VRE16	*E. faecium*	> 256	192	*vanA*	F	66	J12
VRE17	*E. faecium*	> 256	64	*vanA*	F	72	J15
VRE18	*E. faecium*	> 256	192	*vanA*	F	83	J15
VRE19	*E. faecium*	> 256	4	*vanB*	F	79	J12.8

in the isolate and the identification of VRE genotypes that help to confirm whether an isolate has intrinsic (*vanC*) or acquired resistance (*vanA* or *vanB*). The knowledge of the type of resistance is critical for infection control purposes. Isolates with the *vanA* and *vanB* genes are transferable and can spread from bacteria to bacteria and has character of a typical nosocomial infection. These genes are usually coded in the plasmids (Werner et al. 1997; Praharaj et al. 2013). On the other side, *vanC* gene is not transferable; this gene is associated less frequently with serious infections. The levels of antibiotic susceptibility isolates also help to differentiate *vanA* and *vanB* and *vanC* isolates. Rather, a selective pressure exerted by oral vancomycin may facilitate the exogenous acquisition of VRE or transferring vancomycin resistance genes from other species of bacteria to the enterococci in the intestinal tract. Evidence showed that the treatment of CD infection with metronidazole or vancomycin can promote VRE transmission and thus initiate persistent colonization of VRE (Correa-Martínez et al. 2021; Egan et al. 2022). However, it should be noted that the MIC levels of local VRE isolates may varying in the wide range. The classification of genotype based only on susceptibility may lead to a certain percentage of inaccuracy and lead to false positivity and discrepations in the VRE genotypes, especially in *vanB* genotype. The *van* gene, which is frequently plasmid-borne, can be transferred in vitro from enterococci to a variety of gram-positive microorganisms including *Staphylococcus aureus* and/or *Streptococcus* sp., without excluding *Clostridium* sp. (Cattoir and Leclerccq 2012; Faron et al. 2016; Stogios and Savchenko 2020). A parallel VRE infection and CD infection can be potentially fatal for at-risk patients. Infections caused by these pathogens are among the most common and risky diseases and are also included in the ESCAPE/ESKAPE (ESCAPE

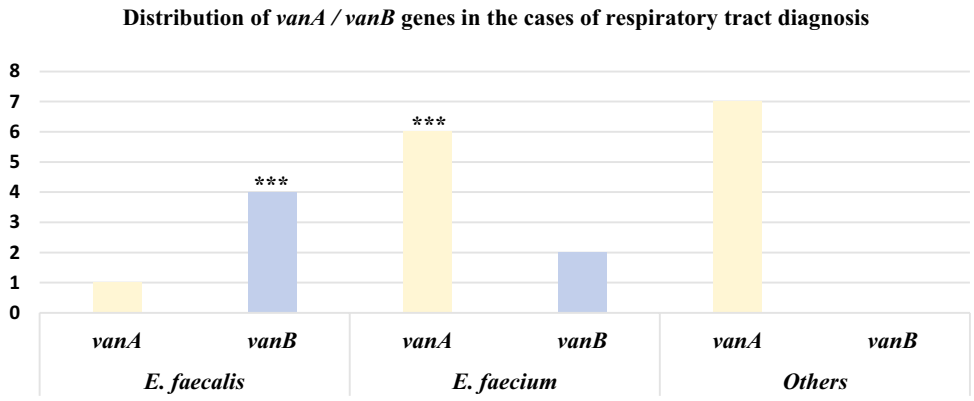

Fig. 4 Distribution *of vanA*/*vanB* genes in cases of diagnosis of the gastrointestinal tract ($p < 0.001$***) (ANOVA, one-way analysis of variance)

Table 8 List of patients with other types of disease or infection (diagnosis based on ICD-10-CM nomenclature) with confirmed VRE isolates (VAN vancomycin, TEI teicoplanin, F female, M male)

Number	Isolates	VAN µg/mL	TEI µg/mL	van genes	Sex	Age	Diagnose
VRE1	*E. faecium*	> 256	192	*vanA*	F	91	E86
VRE2	*E. sulfurous*	> 256	4	*vanB*	F	90	I95.9
VRE3	*E. faecium*	> 256	64	*vanA*	F	78	R50.9
VRE4	*E. faecalis*	192	16	*vanB*	F	84	I63.4
VRE5	*E. faecium*	> 256	> 256	*vanA*	F	82	C67.2
VRE6	*E. faecium*	> 256	> 256	*vanA*	F	91	D50.0
VRE7	*E. solitarius*	> 256	1.5	*vanB*	M	91	R50.9
VRE8	*E. faecium*	> 256	> 256	*vanA*	M	75	A49.9
VRE9	*E. faecalis*	> 256	128	*vanA*	M	73	I63.2
VRE10	*E. faecium*	> 256	64	*vanA*	M	73	I50.9
VRE11	*E. faecium*	> 256	> 256	*vanA*	M	71	R02
VRE12	*E. faecium*	> 256	96	*vanA*	F	67	I63.9

including enterococci and *C. difficile* infections, ESKAPE including enterococci and *Klebsiella* sp. infections) group of pathogens. The risk factors for the development of VRE infection are very similar to those known to develop *C. difficile* infection. That is why we chose this group of patients for the study. Patients positive for *C. difficile* are potentially more at risk of spreading VRE infection (De Oliveira et al. 2020). Other risk factors specifically associated with VRE infections include bacteraemia caused by another pathogen, cancer, acute or chronic renal failure, neutropenia, and prolonged hospitalization.

E. faecium is the most frequently isolated species of VRE in hospitals and build wide range resistance against to vancomycin (> 128 µg/mL) and teicoplanin (≥ 16 µg/mL) resistance (Faron et al. 2016). These isolates typically contain the *vanA* gene. Usually, isolate *vanB* genotypes typically produce a lower level of resistance against to vancomycin (MIC 16 to 64 µg/mL) and are susceptible to teicoplanin (MIC ≤ 1 µg/mL). Based on our analysis, we confirmed *vanB* isolates with susceptibility to vancomycin over than 64 µg/mL and susceptibility to teicoplanin below than 64 µg/mL. These characteristics are indicative for isolates of the *vanA* genotype. Typically, *vanB* genotype isolates produce a lower level of resistance to vancomycin (MIC 16 to 64 µg/mL) and are susceptible to teicoplanin (MIC ≤ 1 µg/mL) (Cetinkaya et al. 2000; Faron et al. 2016). This high level of MIC for vancomycin may be because of the character of our set of patients. All patients had a positive GDH antigen test also for CD toxins A and B, and vancomycin is the most preferred drug to treat CD infections. One of the most relevant step in the development of VRE infections is previous antibiotic therapy with drugs mainly against anaerobic bacteria, and vancomycin is the drug of choice for therapy. Metronidazole can promote the growth of VRE isolates by eliminating the anaerobic microbiota (Reinseth et al. 2020).

However, the administration of vancomycin in combination with third-generation of cephalosporins has been associated with other risk factors for colonization and may be an initial step in initiation and expansion of VRE infection without previous clostridial infection. Enterococci may exhibit the ability to acquire new mechanisms of resistance and have intrinsic resistance, particularly to antibiotics that inhibit cell wall syntheses such as β-lactams (penicillin/ampicillin), aminoglycoside (streptomycin, gentamicin), and glycopeptides (teicoplanin, vancomycin) (Hollenbeck and Rice 2012). The significant part of *E. faecium* isolates reveal resistance to vancomycin and ampicillin, and high levels of aminoglycoside resistance were also observed. Currently, there are a variety of challenges related to VRE infection and

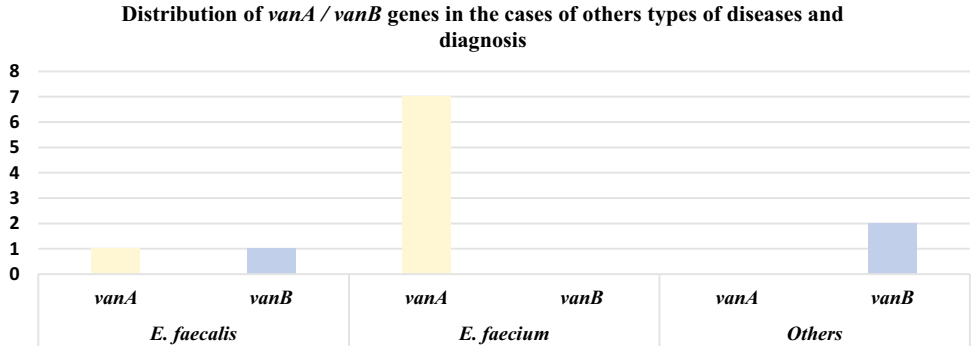

Fig. 5 Distribution of *vanA*/*vanB* genes in the cases of other types of diseases and diagnosis

therapy. The major problems in therapy are high resistance and high tolerance to the most commonly used antibiotic drugs. On the side of resistance, enterococci are intrinsically resistant to the cephalosporin class, semisynthetic penicillin, and oxacillin and also have gained resistance against aminoglycoside (streptomycin, gentamicin) (Miller et al. 2014; Eliopoulos and Gold 2001).

The isolates *E. faecium* and *E. faecalis* are among the most resistant bacteria to antibiotics. Linezolid, an oxazolidinone antibiotic, acts on the principle as an inhibitor of the 50S ribosomal subunit in several gram-positive bacteria, especially resistant organisms, such as VRE, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate (VISA) or -resistant *S. aureus* (VRSA), and penicillin-resistant pneumococci. However, available data from other countries also suggest that some level of resistance to linezolid remains unchanged at a stable level (Krawczyk et al. 2020). Our set of patients was largely composed of patients who has been diagnosed with chronic diseases or other types of disease whose therapy is associated with long-term or/and where repeated administration of antibiotic therapy is necessary.

Conclusion

Enterococci with gained resistance to vancomycin and other glycopeptides have spread rapidly throughout Europe since 1986 (O'Driscoll and Crank 2015). The results of our study indicate the local dominance character of the *vanA* genotype in the hospital. These isolates carry virulent genes that contribute to a high resistance level against to vancomycin, teicoplanin, and occasionally to linezolid. Molecular techniques are highly effective in detecting genes responsible for resistance to VRE, increasing the quality of monitoring and control of the spread of VRE infection in hospitals.

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Declarations

Conflict of interest The authors declare no competing interests.

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